Mechanisms of Microbial Spoilage of Fruits and Vegetables

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15.1 INTRODUCTION

In the various stages of shipment, transshipment, and storage that separate the producers of fruits and vegetables from wholesalers, distributors, and retailers, accumulated losses due to spoilage can, depending on the commodity in question, destroy 25 to 80% of fresh produce before it reaches the consumer (Baldwin, 2001). In recent decades, the technologies available to the fresh produce industries to reduce spoilage have become increasingly sophisticated. Improved understanding of produce storage physiology and response to modified atmospheres, microprocessor-controlled atmospheric and temperature sensors and control systems, and novel approaches to controlling spoilage organisms have improved the overall efficiency of the fresh produce handling infrastructure, but new challenges are emerging. These advances are being applied to a globalized produce distribution network that is offering an increasingly diverse selection of fruits and vegetables, in addition to an expanding range of complex products such as ready-to-eat salads and mixed vegetables (Garrett, 2002). The diversity of the fresh fruits and vegetables available, coupled with the logistical complexity of a globalized network of produce growers, distributors and retailers, make the issue of spoilage a significant economic factor. This chapter will provide an overview of the key issues surrounding microbial spoilage of fresh produce, including the mechanisms by which produce may become infected, the types of microorganisms that cause spoilage of produce, and a presentation of case studies of microbial spoilage of archetypal fresh fruits and vegetables.

15.2 DISEASE VS. SPOILAGE

15.2.1 Abiotic Spoilage and Its Relationship to Biotic Spoilage

Spoilage is a general term that describes a loss of marketable quality of fresh produce (Brackett, 1997). This spoilage may be the loss of appealing qualities, such as aroma (intensity, complexity, etc.), texture (firmness, crunch, mouth feel, etc.), taste (the balance of sweetness and acidity), or appearance (color, evenness, etc.). Spoilage of this type can generally be ascribed to abiotic (or, more precisely, apathogenic) factors, such as the physiological age of the produce, the temperature at which the produce is stored, or the atmosphere mix used in storage. These circumstances alter the physiology of the produce such that marketable quality is lost through chill injury, water loss or some other mechanism. Fruits and vegetables that continue to ripen after having been picked (climacteric fruits) are especially susceptible to this kind of spoilage and have a relatively short shelf life before they become overripe and lose marketable value (e.g., banana, avocado, tomato). Control of atmosphere or use of special edible coatings may help to extend the shelf life of these types of fruits and vegetables (Baldwin, 2001).

It should be noted that fresh produce that is shipped across great distances is vulnerable to spoilage during transit, and greater distances and longer transit times become increasingly more problematic. Produce that is shipped internationally must also withstand required disinfestation procedures between ports of export and import. To accommodate these regulatory and economic factors, loss of a certain amount of marketable quality may be unavoidable. The loss of positive attributes may result in a lower price premium for the produce but does not necessarily result in removal of the produce from the marketplace.

Spoilage may also take the form of the development of undesirable qualities, including off-aromas, off-flavors, or textural and appearance changes such as sliminess, moldiness, blemishes, etc. This type of spoilage typically results from the action of microorganisms such as bacteria and fungi. While the abiotic or physiological spoilage can, in the eyes of the consumer, make "appealing" produce merely "acceptable," microbial spoilage renders produce "unacceptable," with concomitantly greater economic loss to the producer, wholesaler and retailer of fresh produce. In some cases, microbial spoilage can result in vegetable food products that are not merely unacceptable, but harmful, as in the case of mycotoxins such as aflatoxin on grains and patulin on apples.

15.2.2 PREHARVEST VS. POSTHARVEST

Phytopathogens are organisms that cause injury or disease to plants. In a discussion of spoilage, a distinction should be drawn between preharvest diseases of living plants and postharvest diseases of the plant organs that are of economic importance as fresh produce, even though, in many cases, the same phytopathogens cause both classes of disease. A classic example of this is potato late blight disease (*Phytophthora infestans*), the cause of the Irish potato famine of the 1840s. *Ph. infestans* attacks potato leaves and shoots, degrading and decaying the tissues, reducing or eliminating the photosynthetic capacity of the crop. It also causes the harvested potatoes to rot in storage and during shipment and is therefore considered to be a spoilage concern (Niemira et al., 1999). In presenting the distinction between *field* pathogens and *storage* or *spoilage* pathogens (the provinces of *plant pathology* and *food microbiology*, respectively), it should be acknowledged that this is a somewhat arbitrary separation based on the technical factors of produce production and distribution, rather than a distinction that arises from the biology or ecology of plants and their pathogens. Indeed, in many cases spoilage organisms are introduced to the produce during its time in the field but do not cause damage until the conditions of storage and shipment allow them to proliferate. In some cases, field infections with pathogens or symbionts stimulate an induced resistance response that renders the resulting produce less susceptible to spoilage (Tuzun and Kloepper, 1995; Niemira et al., 1996).

Field diseases of crop plants are frequently treated using cultural or agrochemical interventions to exclude, contain, or eradicate the phytopathogens that cause them (Agrios, 1997). Living plants interact with their environment; disease-resistant varieties are able to mount a complex range of native defenses against phytopathogens

(Niemira et al., 1999). In contrast, diseases that occur on the harvested produce, are, by definition, attacking an isolated plant organ. Fresh produce is physiologically alive, in that it is able to exchange water and gas with its environment, mount physiological defenses to attack, and undergo changes in metabolism and physiology. However, the specialized nature of the tissue in question means that the harvested produce can only draw on a more limited range of physiological options, and a more limited metabolic reserve, in responding to attack by phytopathogens (Brackett, 1997). As with field disease resistance, resistance of the produce to spoilage organisms is incorporated in the varieties during the breeding process as much as possible (Niemira et al., 1999). However, unlike field diseases, which act on plants growing outdoors, storage diseases occur under controlled conditions (e.g., storage facility, the packing house, the shipping container, and the wholesale redistribution center). In this context, although varietal- and chemical-based interventions are used, it is the manipulation and control of the environment that becomes the predominant means of preserving produce quality.

15.2.3 Spoilage Organisms

Postharvest spoilage can be caused by a wide variety of different organisms. The proverbial "worm in the apple" is an obvious example of the kind of damage caused by invertebrates such as arthropods and nematodes. Although not the subject of the present discussion, invertebrates such as the carrot root knot nematode (*Meloidogyne hapla*), the green peach aphid (*Myzus persicae*) and the Mediterranean fruit fly (*Ceratitis capitata*) are only a few of the thousands of pests that can lead to spoilage diseases (van Emden et al., 1969; White and Elson-Harris, 1994; Agrios, 1997).

Phytopathogenic fungi and bacteria are varied, physiologically and taxonomically, and often closely related to species that are nonpathogenic, or are pathogenic to organisms other than plants. Of the microorganisms that cause plant diseases, a smaller subset are responsible for microbial spoilage of fruits and vegetables. An abbreviated fungal taxonomy (adapted from Agrios, 1997) is presented below:

1. Kingdom: Protozoa (phagotrophic psuedofungi)
   a. Phylum: Plasmodiophoromycota
      i. Class: Plasmodiophoromycetes (endoparasitic slime molds). Key spoilage genera: *Plasmodiophora, Spongospora*
2. Kingdom: Chromista (phototrophic psuedofungi)
   a. Phylum: Oomycota
      i. Class: Oomycetes (water molds, white rusts, downy mildews). Key spoilage genera: *Pythium, Phytophthora, Plasmopara, Bremia, Psuedoperonospora*
3. Kingdom: Fungi (true fungi)
   a. Phylum: Chitridiomycota
      i. Class: Chitridiomycetes. Key spoilage genus: *Synchytrium*
   b. Phylum: Zygomycota
      i. Class: Zygomycetes (bread molds). Key spoilage genera: *Rhizopus, Mucor*

c. Phylum: Ascomycota
   i. Class: Pyrenomycetes. Key spoilage genus: *Glomerella*
   ii. Class: Loculoascomycetes. Key spoilage genera: *Elsinoe, Venturia*
   iii. Class: Discomycetes. Key spoilage genera: *Monilinia, Sclerotinia, Sclerotium*
   iv. Class: Dueteromycetes: Key spoilage genera: *Penicillium, Aspergillus, Fusarium, Alternaria, Botrytis, Rhizoctonia*

Fungi have been broadly grouped into four classes, according to their phytopathogenicity (Prell and Day, 2000): pure saprophytes (nonpathogenic, lives on dead tissue), opportunistically phytopathogenic saprophytes (attack weakened, stressed, or senescent plants, live on freshly digested tissue), primary pathogens/necrotrophs (attack healthy plants, live on freshly digested tissue), and biotrophs (feed parasitically or symbiotically on living tissue). Of the more than 100,000 known species of fungi, roughly 10,000 species can cause disease in plants; nearly the same proportion of the approximately 1,600 species of bacteria, about 100 species, are phytopathogenic (Agrios, 1997). A relative handful of genera are responsible for the most serious bacterial plant diseases, and a subset of these are most significant from the standpoint of postharvest spoilage of produce: *Clavibacter, Erwinia, Pseudomonas, Xanthomonas,* and *Streptomyces.* Among the species within each of these genera, there is great variation of biochemistry, ecology, and pathogenic significance. For example, some of these, such as the *E. carotovora* group, are capable of producing the pectolytic enzymes that allow them to penetrate plant epidermal tissue, while others, such as the *E. amylovora* group, do not; this is the type of difference that separates primary from secondary or opportunistic pathogens.

In order to improve the clarity and utility of the nomenclature of the phytopathogenic bacteria, the taxonomy has recently been revised to regroup the pectolytic members of *Erwinia* under a new genus, *Pectobacterium*, and also to elevate certain subspecies to species status (Hauben et al., 1998; Gardan et al., 2003). Thus, *E. carotovora* is now more properly referred to as *Pe. carotovorum*; for the purposes of clarity and ease of reference to the literature, the older nomenclature will be used in the remainder of this chapter when referring to the pectolytic members of *Erwinia*.

15.3 EARLY SPOILAGE PATHOGENESIS EVENTS: OVERVIEW

15.3.1 Plant Defenses

Spoilage disease occurs at the intersection of the factors that make up the disease triangle (Figure 15.1). The first element of the triangle is the host plant, or, more specifically, the plant part that is of value as a produce commodity. Plants use a variety of mechanisms to defend against pathogen attack. These complex and highly evolved defenses may be a preexisting part of the plant's normal state, such as the anatomical leaf structure that isolates the cells from the environment (Figure 15.2). The outer, waxy layers of the epidermis form the bulk of the cuticle and are known as cutin (Figure 15.3). The complex waxes of the cutin and the additional pectin, cellulose, and hemicellulose structures are resistant to physical or chemical degradation, although

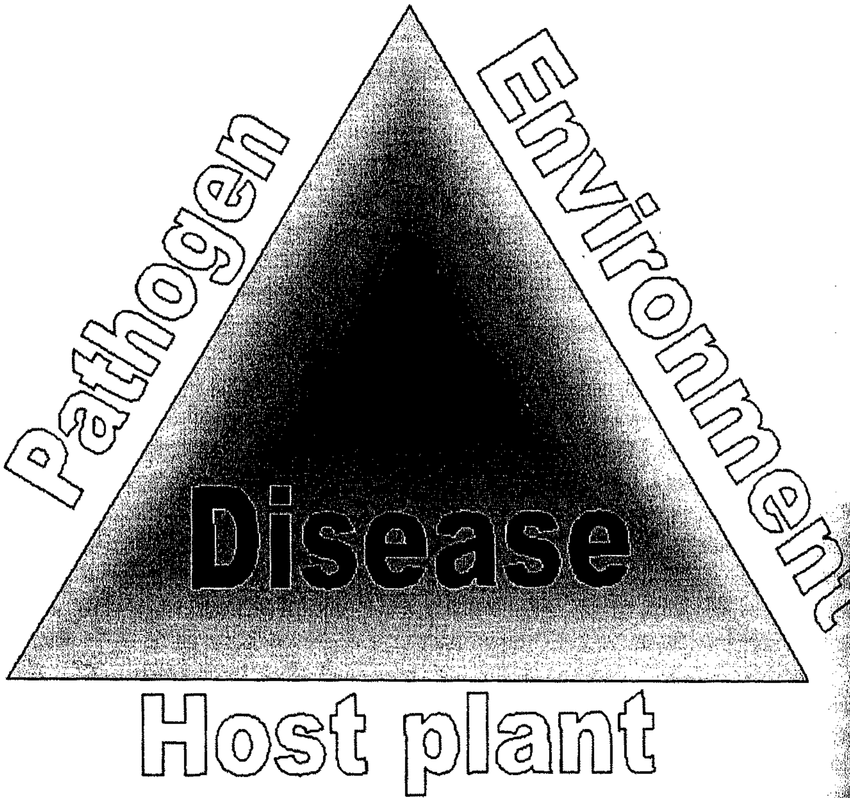

FIGURE 15.1 The plant pathology "disease triangle." Disease occurs when a compatible pathogen and host are together under the proper environmental conditions.

enzymatic degradation by capable phytopathogens is a weakness. Other forms of defense may consist of constitutively produced biochemicals, or responses that are activated upon pathogen attack, such as hypersensitivity responses and induction of phytoalexins and/or pathogenesis-related proteins (PRP). Induced resistance responses can, in some cases, persist and act as a form of "immunization" of the plant against subsequent pathogen attack in the field and/or in storage (Tuzun and Kloepper, 1995; Niemira et al., 1996). Disease-resistant varieties have been bred to possess systemic and/or inducible defenses against a particular pathogen. This varietal resistance may be of the broad/horizontal type (somewhat resistant against all races/genotypes of a particular pathogen), or it may be narrow/vertical (completely resistant to certain races/genotypes of the pathogen, completely susceptible to others). Unfortunately, the biochemical details of disease resistance are often only poorly understood, and the ability of one plant organ (e.g., leaves) to resist pathogen attack does not necessarily correlate with the disease resistance of another (e.g., tubers) (Niemira et al., 1999).

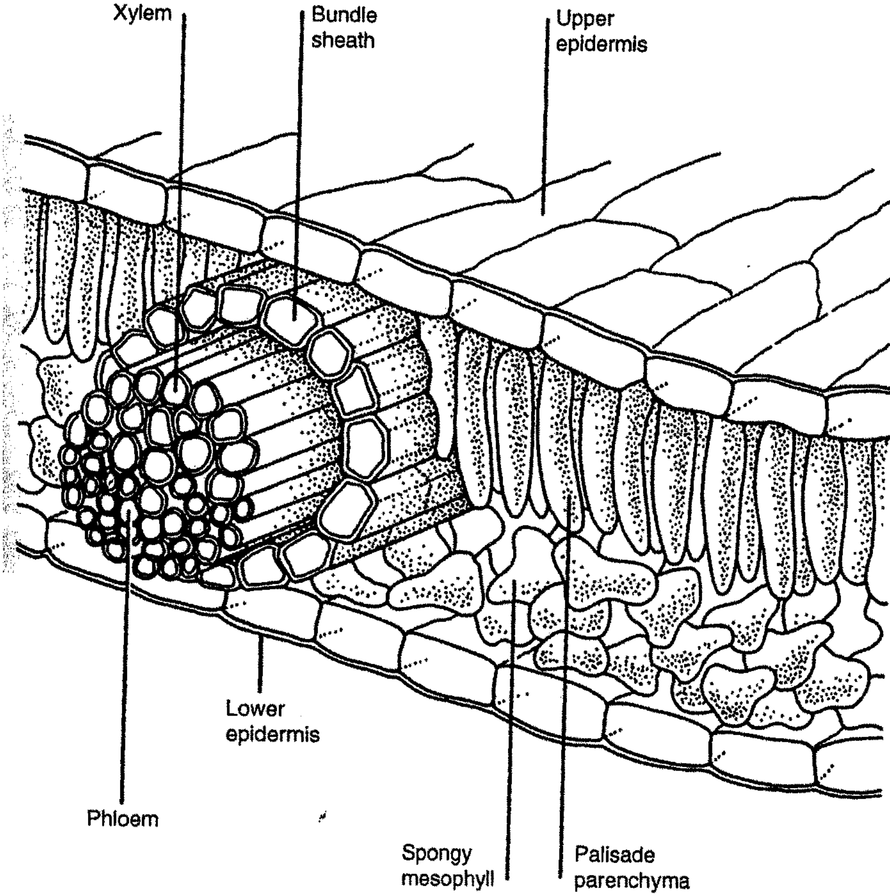

FIGURE 15.2 Cross-sectional anatomy of leaf tissue. (From Mauseth, 1988. With permission.)

15.3.2 Pathogen Attack

The second element in the progression of microbial spoilage is the introduction of a compatible pathogen to the produce. Bacterial and fungal phytopathogens can be introduced to fruits and vegetables at almost any point during the growth, maturation, harvest, storage, or shipment of the produce by exposure to contaminated water, dust, mechanical equipment, etc. (Beuchat, 1995). As previously indicated, the surfaces of fruits and vegetables have evolved to present as impervious a barrier as feasible, with multiple layers of chemical and mechanical defenses. Thus, while the first step in spoilage is getting the pathogen *onto* the produce, the second, more important step in spoilage is getting the pathogen *into* the produce. Obligate phytopathogens have evolved a suite of tools that allows them to penetrate the plants' defenses. These may take the form of specialized physical structures, such as fungal appressoria (Figure 15.4), in addition to specialized enzymes that can degrade the

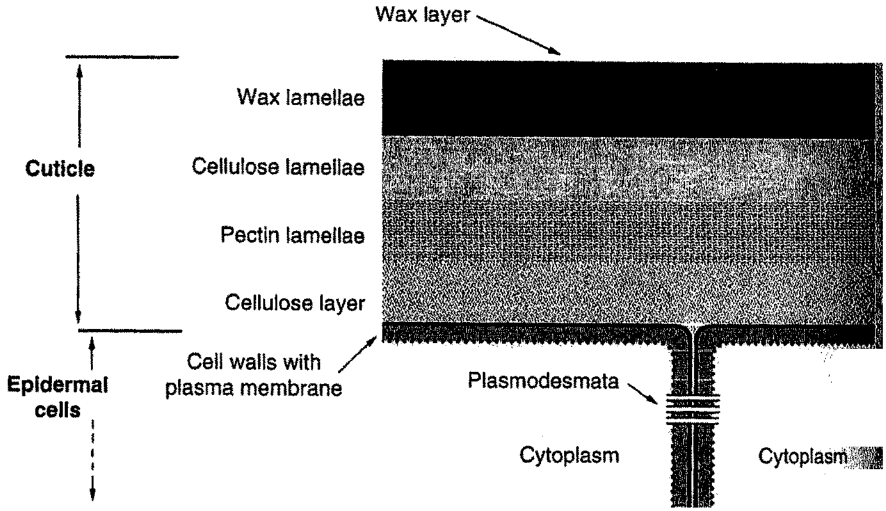

FIGURE 15.3 Diagrammatic representation of foliar epidermal cells and associated cuticle layer.

cellulose, hemicellulose, pectin, and cutin of the epidermal and endodermal tissues (Figure 15.5). While cellulases are of notable importance with regard to field diseases, they are considered to be relatively less significant than pectinases with regard to storage diseases (Brackett, 1997).

Many damaging forms of spoilage are caused by organisms that, by themselves, are unable to penetrate the epidermis of the produce. These organisms, often referred to as secondary or opportunistic pathogens, rely on a breach of the surface integrity to gain entry to the inner tissues of the leaf, fruit, tuber, etc. This opening may be a naturally occurring anatomical structure opening such as a stomate, hydathode, stem end scar, etc., or it may be a breach caused by an obligate pathogen, a pest (nematode, insect, etc.), or some other biotic agent (Figure 15.4).

Frequently, the breach is of abiotic origin, such as a puncture, fracture, abrasion, or some other wound resulting from mishandling at some stage of the production cycle. Poorly designed or maintained equipment can cause these wounds at a variety of stages during the growth, harvest, washing, packing, or shipping of the produce. Produce that has been harvested by cutting or has been prepared by cutting, sectioning, peeling, chopping, or otherwise treated in some way that breaches the epidermis is therefore especially vulnerable to spoilage. The complexity of the physiology and microbiology of processed fruit and vegetable products, including those with more than one vegetable component, warrants a fuller discussion than can be presented herein. Many of the food quality and food safety issues related to minimally processed and/or fresh-cut fruits and vegetables have been recently reviewed (Garrett, 2002; Zhuang et al., 2003).

Two of the most commonly destructive bacteria of stored produce are *E. carotovora* pv. *carotovora* and *Ps. fluorescens* (Agrios, 1997). These are responsible for the soft rot of a wide variety of fruits and vegetables in storage; these pathogens

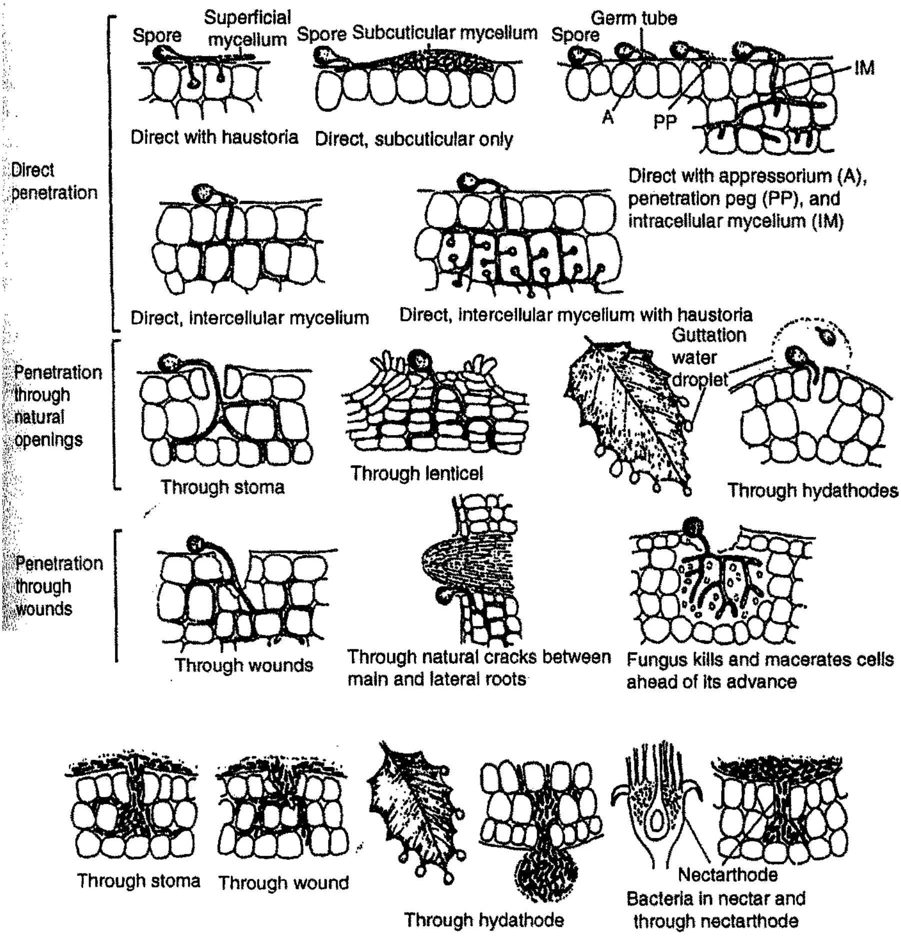

FIGURE 15.4 Various methods of penetration of plant tissue by phytopathogenic fungi (top three rows) and bacteria (bottom row). (From Agrios, 1997. With permission.)

will be cited repeatedly later in this chapter, as part of the case studies of key fruits and vegetables. Stored produce that is clean from the field may still become infected by contact with contaminated surfaces (equipment, hands, detritus, etc.), and via infiltration or absorption of contaminated wash water. Wounds provide common entry points, and the bacteria multiply in intercellular spaces. Produce that is under attack by soft-rotting bacteria can quickly degrade, such that by the time a soft-rot infection is apparent by appearance or by a characteristic smell the shelf life of the produce can be counted in days, if not hours. In the initial phase of infection by *Erwinia*, the bacteria reproduces on the surface and internally without digesting the polysaccharide matrix of the cell wall; when the population density reaches a critical level, a quorum-sensing pathway is initiated. Acyl-homoserine lactone (AHL) is a signal molecule that initiates the production of pectolytic enzymes that release oligo- and monosaccharides on which the bacteria feed, degrading the plant tissues in the

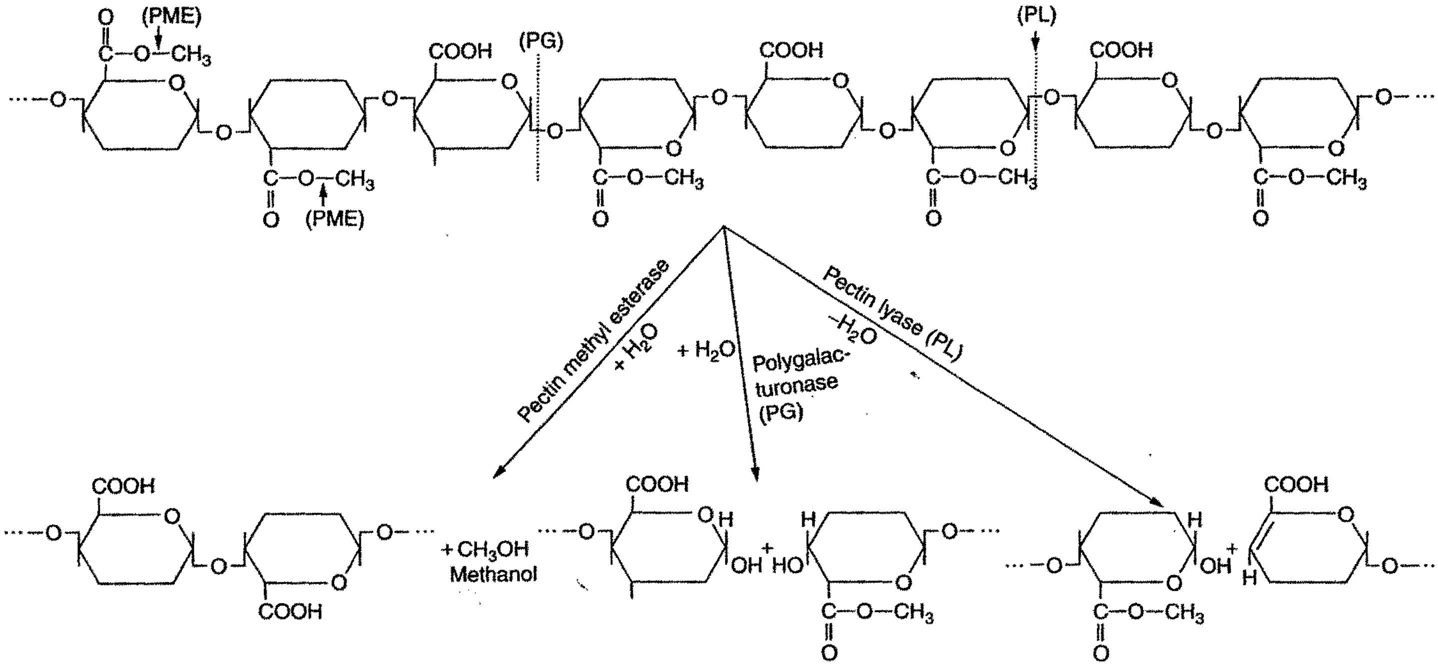

FIGURE 15.5 Pectin degradation by pectin methyl esterase, polygalacturonase, and pectin lyase. (From Agrios, 1997. With permission.)

process (Dong et al., 2001; Leadbetter, 2001). The bacteria multiply rapidly in the sugar- and nutrient-rich medium of the degraded tissues, and, because the bacteria are already at elevated population levels before the tissue degradation triggers plant defense responses, these responses can be quickly overwhelmed. Given the speed and destructive power of bacterial soft rots, it is generally accepted that the control of bacterial soft rot is best accomplished by exclusion and sanitation. The ultimate goals are to prevent the initial infection of the produce, and, through closely controlled conditions in storage, to prevent the growth of the bacteria.

15.3.3 Disease-Conducive Conditions

The final element of the disease triangle is the environment in which the host and pathogen interact. When speaking of phytopathogenesis in general, it is difficult, if not impossible, to make sweeping statements regarding the conditions under which phytopathogens can potentially cause disease, given the diverse nature of the pathogens, their plant hosts, and environmental variations they experience. However, in a discussion concerned more narrowly with postharvest spoilage, the only relevant microclimate conditions are predetermined by (1) the produce in question and (2) market forces of time, geography, and economics. According to the (arbitrary) distinction previously described, a pathogen that is introduced to the produce is relevant to this discussion only if it begins to cause, or continues to cause, disease in the postharvest environment. Therefore, the effects of environment are more easily addressed, because they are artificially created and maintained. Modern storage and shipment/transshipment facilities maintain produce in conditions that are designed to preserve freshness and marketability. Typically, this means circulating air maintained at 5 to 7°C, with relatively high humidity (90%+). Air circulation helps to prevent standing water droplets or films on the surface of the produce and also assists in removing the heat generated by the respiring produce; this is especially important for produce that will be stored for extended periods of time.

15.4 CASE STUDIES OF KEY PATHOGENS

15.4.1 Categories of Produce

With regard to potential for spoilage, the thousands of commercially available varieties of fresh fruits and vegetables may be arbitrarily grouped in a number of ways. A strict botanical grouping would separate fruits (e.g., melon, tomato) from leaves (e.g., lettuce, cabbage) from roots (e.g., carrot, sweet potato) from tubers (e.g., potato), etc. A physiological grouping might draw a distinction between climacteric (e.g., banana, tomato) and nonclimacteric produce (e.g., strawberry, orange). A focus on the potential for field exposure to soil-borne phytopathogens might distinguish arboreal (e.g., apple, tomato) from terrestrial (e.g., squash, melon) from subterranean (e.g., carrot, potato). The potential economic impact of spoilage may prompt a grouping based on market value, separating relatively low value (e.g., apple, carrot, potato) from relatively high value (e.g., blueberry, tomato, asparagus). Given the potential for transmission of unwanted phytopathogens presented by workers and equipment, a distinction can be drawn based on hand-harvested (e.g., melon, tomato) and semimechanized (e.g., cherry, orange) and mechanized (e.g., carrot, potato). Historically, seasonal variation in storage condition influenced the spoilage of spring (e.g., peas, rhubarb) vs. summer (e.g., blueberry, cucumber) vs. fall (e.g., apple, potato) crops. A distinction could be drawn between mechanized, high-input agriculture, and low-input or organic practices. The geographical source of the produce has a significant impact on the potential for spoilage; locally grown produce is handled less and spends less time in storage than produce grown regionally, shipped from across a continent or imported from distant locales. Of the various factors that could be considered, the issue most significant in a globalized market with produce shipped long distances, the relative amount of time spent in storage and therefore the length of the window of opportunity for a spoilage microorganism to act, will be the initial grouping factor used herein. Relatively perishable commodities, those that spend relatively short periods of time in storage, will be grouped separately from relatively storable commodities. Within these groups, arbitrary subgroups based on gross structure will be established.

Each of the fruits and vegetables that will be considered can fall victim to a number of phytopathogens, and, conversely, a given pathogen may have a wide host range. Certain common themes will become apparent in the ways in which spoilage develops among the various commodity/pathogen combinations, yet each disease progression has unique aspects that are instructive to consider. Note that with regard to nomenclature many diseases are referred to by the genus of the responsible pathogen (e.g., "Fusarium rot" is caused by *Fusarium* spp., while in other cases the genus may be a synonym for another common name of the disease, e.g., "*Botrytis*" is equivalent to gray mold, caused by *Botrytis cinerea).*

15.4.2 Perishable Produce

15.4.2.1 Leafy Produce

Botrytis cinerea is a fungus that attacks a wide range of fruits and vegetables and is the cause of gray mold of leafy vegetables such as lettuce, endive, and cabbage (Anon., 2000). *Botrytis* establishes itself in the inner leaves of the head, and the mycelium can spread to encompass the entire leaf. Under conducive conditions, the inner core of the head may be completely engulfed in mycelium with little external sign of disease. Initial infection typically occurs when the ambient temperature in the leaf canopy is less than 25°C; thereafter, fungal growth can continue at temperatures ranging from 0 to 35°C. The fungus can grow under refrigeration temperatures (0 to 10°C), and therefore presents a particular problem in storage for produce that was infected relatively close to harvest. When infecting leaves, *Botrytis cinerea* can express cutinases, pectinases, and cellulases to digest the leaf tissue to component oligo- and monosaccharides (Carlile et al., 2001). The degraded leaf tissue resulting from the growth of *Botrytis* can lead to secondary infection by other fungi or by bacterial pathogens, such as *E. carotovora*, which accelerates the extent of spoilage.

Downy mildew of lettuce, caused by the oomycte *Bremia lactucae*, is similar to *Botrytis* in that, following inoculation in the field, subsequent spoilage during storage or shipment can approach 100% (Raid and Datnoff, 1992). This pathogen is typically most active under conditions of high relative humidity, and when a thin film of water is persistent on the leaf surface. Sporangia are deposited on the leaf surface, and a germ tube grows across the leaf, penetrating stomata. In susceptible varieties, initial infections are visible as small spots, typically on the lower leaf surface. While these lesions can expand into the inner leaves, the outer leaves are most susceptible to expanding chlorosis, browning and necrotic streaking, with the necrotic tissue serving as a gateway for other pathogens such as *Botrytis* or bacterial pathogens.

15.4.2.2 Cruciferous Produce

The fungal pathogen *Alternaria brassicae* attacks cabbage, broccoli, and cauliflower. Infection of mature plants typically occurs in the field, where conidia are dispersed by wind and water, although postharvest infection can also be a problem. High levels of humidity or water films on produce are required for infection (Humperson-Jones and Phelps, 1989). Older, senescing tissue is typically the part of mature plants most susceptible to *A. brassicae*. After germination, fungal hyphae can penetrate through stomata or wounds and pervade the vascular system, causing streaking, spotting and wilting throughout the plant. Early in the season, this can result in loss of the entire plant as the stem rots; in terms of postharvest spoilage, the damaged outer leaves of cabbage are no longer marketable and must be removed, while the florets of broccoli and cauliflower turn brown, reducing market value. While *A. brassicae* infections are readily managed by fungicide applications and sanitation controls, this pathogen is ubiquitous and a perennial problem for growers and shippers. The diseased tissue can be a serious problem, in that it can provide a breeding ground for other, more aggressive and less readily controlled fungal or bacterial pathogens.

15.4.2.3 Fibrous Produce

Asparagus spears are harvested by cutting in the field; additional trimming is performed in packing sheds and storage facilities. Trimmed spear ends and other plant detritus can accumulate on the packing line, providing a breeding ground for bacteria; contaminated knives, wash water and cooling water can be vectors of soft-rot bacteria such as *E. carotovora*. Asparagus is therefore at a greater risk from cross-contamination than is produce that is harvested whole and intact. Asparagus is also a relatively high-value commodity, which increases the economic impact of superficial or cosmetic loss of quality. Soft-rotting bacteria such as *E. carotovora* and *Pseudomonas* spp. can cause darkened, slimy, sunken spots to appear at the cut base end or at the tips by enzymatic degradation of the tissues. Modified atmosphere packaging that uses an elevated CO_2 level can suppress bacterial soft rots in refrigerated storage (Anderson and Tong, 1993).

Spoilage of celery is frequently caused by grey mold (*Botrytis*), watery soft rot (*Sclerotinia* spp.) and/or bacterial soft rot (*Erwinia* and *Pseudomonas*) (Gross et al., 2002). *Sc. sclerotiorum* and *Sc. minor* can survive in field soils in infected plant detritus for multiple seasons, leading to soil-, water- and wind-borne contamination of stems (Laemmlen, 2001). *Sclerotinia* establishes itself on senescent tissue and spreads mycelium into succulent tissues, using a complex suite of enzymes, including several different polygalacturonases, to break down pectins as it grows (Martel et al., 1996). As the celery stalk tissue is consumed, cottony mycelium may appear on the surface, and ascospore containing sclerotia form. Initial infection is favored by relatively warm conditions (~25°C), but once underway, growth of the fungus can continue at refrigeration temperatures (Agrios, 1997). As with many spoilage conditions previously discussed, the digested and partially digested plant tissue can encourage the growth of other phytopathogenic fungi or bacteria.

15.4.2.4 Soft-Skinned Produce

The growth of *Botrytis* on grape clusters leads to gray mold, also know as bunch rot. Infection frequently occurs in the field, leading to the establishment of a fungal culture within the bunch, in the relatively high-humidity center. When the grape bunches are harvested and stored, the fungal mycelium penetrates the mature grapes and spreads throughout the bunch, leading to a cottony mass that pervades all or part of the bunch (Cappellini et al., 1986). Following the enzymatic degradation of the bunched grapes, secondary fungal or bacterial pathogens may also grow on the stressed and damaged fruit, leading to further discoloration and loss of turgor. The high humidity within a closed plastic bag provides a good environment for Botrytis to grow rapidly; current practice is to use open or perforated bags for table grapes.

As the grape skins lose integrity, water loss may cause them to shrivel. For this reason, *Botrytis* may, in some cases, be intentionally allowed to persist in the field to infect grapes that are intended for processing into some sweet wines. The water loss resulting from the *Botrytis* infection (in this context sometimes referred to as "the noble rot") concentrates the sugars and acids of the grape, resulting in a complex and valuable wine. However, the infection can grow out of control under the wrong environmental conditions, and *Botrytis*-infested detritus can be a source of inoculum for subsequent or adjacent crops (Bachman, 2001). This risk, and the increased problems associated with spoilage of the *Botrytis*-infested bunches before processing, or of cross-contamination of other grapes, particularly table grapes, via contaminated workers and equipment make this a somewhat inconsistently effecacious management strategy.

The tomato is susceptible to a wide variety of spoilage pathogens, including (but not limited to) fungal rots caused by *Alternaria alternata*, *Colletotrichum phomoides*, *Fusarium* spp., *Phytophthora* spp., and *Sclerotinia* spp., as well as bacterial soft rots caused by *Erwinia*, *Pseudomonas* and *Xanthomonas campestris* (Gross et al., 2002). Infection of the tomato fruit with spoilage organisms can occur in the field but also results from improper handling during harvest and shipment. Vine-ripened tomatoes are easily damaged and must be handled with care to avoid inoculation with phytopathogens. Although the flavor and desirability (and therefore the market value) of vine-ripened tomatoes is typically higher than that of tomatoes that are picked unripe, the latter are firmer and more resistant to bruising and are better able to withstand handling and can therefore be shipped and packaged more easily. However, the relative durability of the unripe fruit means that it is more likely to receive rougher treatment, which can crack, puncture, crush, or otherwise damage the skin.

Accumulated detritus from damaged fruit on packing and washing equipment, shipping containers, and line workers can introduce spoilage organisms to the produce. Tomato anthracnose, also called ripe rot, is caused by *Colletotrichum phomoides*. The growth of this fungus is favored under relatively warm conditions with high humidity, such as those found late in the season. It can lead to severe postharvest storage losses in most tomato varieties, but particularly in canning varieties (Agrios, 1997). As the mycelium penetrates the tomato tissue, small, sunken, water-soaked spots appear on the surface. These blemishes enlarge to cover an appreciable part of the tomato surface, merge, and ultimately lead to a watery softening and collapse of the fruit as the mycelium grows throughout the tomato fruit. Secondary infection by bacterial pathogens is common once the blemishes begin to merge, rapidly accelerating the spoilage of the produce. Control of this disease centers around exclusion and sanitation.

15.4.2.5 Firm-Skinned Produce

Citrus fruits such as oranges, lemons, and limes are susceptible to blue molds and green molds caused by *Penicillium* spp. Fractured, punctured, or otherwise wounded fruit is most vulnerable to infection, but otherwise healthy fruit may become infected via direct hyphal growth if left in contact with diseased fruit (Agrios, 1997). *Penicillium* rots on citrus fruits first appear as small, water-soaked discolorations. The fungus produces pectinases and cellulases to digest and invade the plant tissue (Ribon et al., 2002). A visible, cottony white mold spreads across the surface of the fruit, and sporulation takes place at the center of the infection, giving the characteristic blue or green color.

Injury, such as that resulting from mishandling, can lead to a variety of infections of firm-skinned melons such as cantaloupe, watermelon, and honeydew. Even in the absence of any injury, however, healthy melons are susceptible to fungal rots caused by *Fusarium* spp. and *Phytophthora* spp. and associated bacterial infections by *Erwinia* and *Pseudomonas* (Roberts et al., 2001). Ripe watermelons can become infected with *Phytophthora capsici* from excessively moist soils in the field or by contact with contaminated equipment or personnel. The fungus can survive in the field in the absence of a host for several seasons. Infectious soils can be carried into previously clean fields on agricultural equipment. *Ph. capsici* fruit rot begins as a small brownish discoloration, circular or oval in shape. These lesions expand rapidly, leading to a darkened, water-soaked appearance of the fruit. As the mycelium invades and digests the rind and interior of the melon, the center of the lesion can become visibly moldy as the fungus sporulates. Once the rot is established, the entire fruit may be consumed within a matter of a few days by the *Phytophthora* alone or in conjunction with other fungal or bacterial pathogens.

15.4.2.6 Berries

Strawberries are picked nearly fully ripe and are easily damaged. The warm, moist conditions of the harvest period, coupled with the growth habit of the strawberry (low to the ground), make soil and water contamination key risk factors. The most common spoilage pathogens on strawberries are grey mold (*Botrytis*) and Rhizopus rot (*Rhizopus stolonifer*) (Gross et al., 2002). The growth and development of these molds on strawberries are typically exacerbated by the easily damaged nature of the product. Strawberries are packed in small containers to avoid crushing and bruising to the greatest extent possible, and although these containers are typically perforated or vented, even slight damage to the berry surface can lead to excessive moisture and/or free fluid accumulation in the container. Once established at warmer temperatures (~25°C), *Botrytis* can continue to grow at refrigeration temperatures, penetrating the fruit and digesting the sugar-rich tissues (Sommer et al., 1973). The close contact of one fruit with another makes possible direct hyphal growth, ultimately leading to a cottony mycelial mass that encompasses the available fruit. As with the development of this pathogen on other commodities, the partially digested fruit tissue supports the growth of secondary spoilage pathogens.

Raspberries are one of the most fragile and easily damaged fruits on the market. They are handled and packaged much the way strawberries are (although with an even greater degree of care) and are similarly susceptible to *Botrytis* and *Rhizopus stolonifer* (Gross et al., 2002). As with *Botrytis*, *Rhizopus* is frequently inoculated onto the fruit while in the field and can lead to many of the same symptoms: extensive hyphal growth and penetration of the fruit, enzymatic digestion of the tissues, envelopment of the available fruit in a cottony mycelial mass, and opportunistic infection by other fungi and bacteria. However, while *Botrytis* can continue to grow (albeit slowly) at temperatures as low as 0°C, *Rhizopus* ceases development below 5°C. The primary means of control of this fungus is rapid chilling of the fruit to < 5°C and consistent temperature control throughout the handling chain.

15.4.3 Storable Produce and Key Spoilage Fungi and Bacteria

15.4.3.1 Roots and Tubers

The major storage pathogens of carrots are, by now, familiar: *Botrytis*, *Erwinia*, *Pseudomonas*, and *Sc. sclerotiorum* (Gross et al., 2002). Unlike the commodities considered to this point, carrots are a subterranean crop and are therefore presented with a combination of factors that serve to increase the risks of inoculation and subsequent spoilage. Crops that suffer from a high degree of field disease are unlikely to be harvested or brought into storage. Therefore, from the standpoint of postharvest spoilage, the greatest spoilage risk factor is the commingling of slightly diseased produce with healthy produce in the storage facility. Carrots and other subterranean crops are more exposed to soil-borne pathogens than are surface-growing or aerial crops. Harvesting of subterranean crops necessarily requires digging, pulling, lifting, or otherwise extracting the produce from the soil, with all of the potential for equipment-related injury. In preparation for market, carrots are topped, that is, the greens are removed; in general, procedures that involve cutting the produce provide an additional avenue for spoilage. Also, the need to wash soil from the carrot requires a more physically aggressive series of handling steps than is required for other commodities. Thus, while the potential for cross contamination via wash water is a common theme for many fruits and vegetables, the intensive scrub process that carrots undergo can lead to greater damage to the surface, providing wounds as entry points for the spoilage organisms. Careful handling during harvest, and exclusion of the pathogens from the growing and packing environment, are the only effective means of control for these pathogens.

Major storage diseases of potatoes include Fusarium rot (*Fusarium* spp.), pink rot (*P. erythroseptica*) and, most notably, late blight (*P. infestans*). *Phytophthora* spp. are field pathogens of most major crop plants. *P. infestans* is the most famous and widely studied oomycete in the history of plant pathology (Kamoun, 2003). This aggressive fungus, responsible for the Irish potato famine of the 1840s, has reemerged in recent years to become the single most devastating pathogen of potatoes in the field and in storage, causing losses as high as US$5 billion annually (Kamoun, 2003). Spores are typically soil- or water-borne but may also be spread via air or by contact with diseased detritus in the field, on equipment, or in storage. Upon germination, fungal hyphae invade and digest tuber tissue, entering via wounds or stomata on the tuber surface. A significant alternate route of infection is via the stolon by which the tuber is connected to the parent plant in the field. Lesions appear as dark brown or purplish blotches, which soon turn sunken and water-soaked (Niemira et al., 1999). The fungus pervades the outer vascular ring of the tuber first, and the entire surface of the tuber may be mottled and diseased before the fungus extends into the interior. Infection with *P. infestans* is typically soon accompanied by growth of secondary soft-rotting pathogens; the disease complex of fungus and bacteria quickly destroys the original diseased tuber and any previously healthy tubers that it contacts, producing an unforgettably putrid characteristic odor. During the harvest process, the need to wash soil from the potato tubers can lead to wounding of the surface and cross contamination among the tubers as they are placed in storage via wash water or contaminated equipment. Intensive chemical applications are required to control the development of *P. infestans* on foliage in the field; sanitation and exclusion are the only means to control this pathogen in storage.

15.4.3.2 Bulbs

Bulb crops such as onion and garlic suffer from spoilage by blue molds (*Penicillium* spp.) and black molds (*Aspergillus niger*), and, more so in the case of onion, concomitant or subsequent growth of *Botrytis* and bacterial soft rots. These pathogens colonize and grow best under conditions of high moisture and/or free water on the bulbs; the growth of these pathogens can be effectively suppressed by reduction of the relative humidity to 60 to 70% (Gross et al., 2002). For many of the commodities considered to this point, low humidity causes an unacceptable degree of wilting, cracking, shrinkage, etc. and loss of marketable quality. However, the bulbous nature of onion and garlic allow them to tolerate these levels with little economic impact, providing a relatively straightforward means of controlling the spoilage (i.e., harvest procedures that keep the bulbs as dry as possible, and low humidity in storage).

15.4.3.3 Soft-Skinned Produce

Apples are most commonly spoiled by *Penicillium* molds (chiefly *Penicillium expansum*), with significant spoilage also caused by gray mold (*B. cinerea*) and bitter rot

(*Colleotothrichum* spp.) (Pierson et al., 1971; Sanderson and Spotts, 1995). *Colleotothrichum* is an imperfect fungus that causes anthracnose diseases of numerous crops (Agrios, 1997). *Colleotothrichum* typically infects apples late in the season, causing loss of fruit in the field and spoilage in storage. Fungal conidia germinate on the apple surface and the hyphae invade the fruit tissue. The circular areas of infected tissue appear sunken and brownish, and within these areas form cushion-like masses of developing fungal conidia called acervuli. The rot spreads toward the center of the fruit, making the apple bitter. The diseased spots grow until they fuse, and, unlike the watery rot that this fungus causes in tomatoes, apples become mummified and infectious with fresh conidia (Agrios, 1997). As with tomatoes, the only practical control for *Colleotothrichum* is exclusion of infected fruit from the storage facility.

15.4.3.4 Firm-Skinned Produce

Despite the physically tough, thick, and waxy rind that characterizes pumpkins and other squash such as the acorn, butternut, crookneck, Hubbard, and winter squash, these cucurbits are vulnerable to a number of spoilage fungi, with the risk of damage proportional to the time in storage. Fungi such as *Aspergillus, Colleotothrichum orbiculare, Rhizopus, Sclerotinia sclerotiorum,* and *Fusarium* are able to infect the squash via natural openings, such as the stem scar and blossom end, but wounds are the most significant means of entry (Zitter, 1992). Infection of the squash typically occurs in the field, prior to harvest, or during the harvest and storing process. Mechanical damage to the rind can arise from rough handling, but also from improperly stacking squash too high, resulting in bruising and cracking. Following germination of the spores of *Fusarium* on the surface or within a wound, the hyphae invade the plant tissues in a familiar sequence of events; the resulting rot tends to be drier and more corky in appearance than many other fruit rots. Brown sunken spots on the surface of the fruit spread more slowly than the internal growth of the fungus suggests. Extensive Fusarium rot manifests as a whitish pink or yellow mold on the surface of the fruit, and colonization of the rotted fruit by secondary bacterial pathogens is likely (Agrios, 1997).

15.5 CONCLUSIONS AND SUMMARY

The spoilage organisms examined in this chapter form a complex, overlapping ecology. For each of the commodities discussed, multiple pathogens operate in concert to attack, feed, and grow, helping each other to accelerate the spoilage of the stored produce. For many pathogen complexes, a reasonably accurate, though simplistic, summary is that a fungus opens the door and *Erwinia* walks in. Exclusion of the pathogens from the field, harvest, and storage is the most straightforward means of controlling these pathogens. For most crops, this means adherence to crop rotations, fungicide application programs during the growing season, and use of resistant varieties. Specific agricultural practices can further reduce the risk of establishment and development of spoilage pathogens. Examples of these would be minimizing the use of wash water as produce is going into storage, to reduce the presence of free water and water films; proper care and handling to avoid mechanical damage to the produce; and close control of temperature and humidity to suppress pathogen development while preserving product quality. The single most important factor is sanitation of the harvest and packing equipment and environment. The removal of pathogen refugia such as plant detritus, and avoiding cross-contamination vectors such as recycling wash waster, poorly designed equipment or poorly trained personnel are the first lines of defense against spoilage microorganisms.

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